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In Vitro Investigation of the Anti-Osteoarthritic and Anti-Inflammatory Properties of Nyctanthes Arbor Tristis Linn Emulgel

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Abstract- This study evaluated the cytotoxic and anti-inflammatory properties of an Emulgel formulated using the leaves of Nyctanthes arbor-tristis. The study found that the Emulgel had no cytotoxic effect on RAW 264.7 cell lines at concentrations of 5mg/mL. The anti-inflammatory properties of the Emulgel were assessed by measuring the release of IL-6 from the treated cells, with Formulation 1 showing the potential as an anti-inflammatory agent. The Emulgel was evaluated for its organoleptic properties, homogeneity, pH determination, spreadability, viscosity, extrudability, in vitro drug release, and stability studies. The phytochemical screening confirmed the presence of alkaloids, carbohydrates, saponins, glycosides, steroids, phenols, and flavonoids in the extract of the leaves. The evaluation parameters for the Emulgel showed that Formulation 2 consistently performed well and was the best among other formulations. The stability studies indicated that Formulation 2 was stable and maintained its physical appearance, spreadability, pH, and drug content parameters over a 3-month period at various temperatures. Future research may involve in vivo testing of the formulation in an osteoarthritis model.

Keywords - Nyctanthes Arbor Tristis Linn Emulgel, Anti-Inflammatory Emulgel, Emulgel, Anti-Osteoarthritic Emulgel

Introduction

Osteoarthritis (OA) is one of the most prevalent musculoskeletal disorders affecting millions of people worldwide, especially those in older age groups. It is a chronic condition that typically results from a combination of biomechanical, genetic, and metabolic factors, leading to the degeneration of the articular cartilage, subchondral bone, synovium, and other joint structures. As the disease progresses, patients experience chronic pain, joint stiffness, decreased mobility, and reduced quality of life [1].

In addition to structural changes, OA is also associated with a persistent low-grade inflammatory response, characterized by the release of various inflammatory cytokines, chemokine's, and growth factors. This inflammatory response further exacerbates joint damage by promoting cartilage degradation, bone resorption, and synovial inflammation. As such, anti-inflammatory therapies have become a focus of research for OA management [2].

Current OA treatments, including NSAIDs and DMOADs, are not always effective, and their long-term use is associated with several adverse effects, such as gastrointestinal ulcers, cardiovascular complications, and renal toxicity. Therefore, there is a need for safe and effective alternative therapies [3].

Nyctanthes Arbor Tristis Linn, commonly known as night-flowering jasmine, is a plant widely distributed in Asia, known for its medicinal properties. The plant has been traditionally used to treat various ailments, including inflammation, fever, arthritis, and other inflammatory conditions. Several studies have reported the anti-inflammatory and analgesic properties of Nyctanthes Arbor Tristis Linn in various in vitro and in vivo models [3].

Emulgel is a topical dosage form that combines the advantages of a gel and a cream, making it an attractive vehicle for delivering drugs to the skin. The formulation can provide prolonged release, increase skin permeation, and reduce side effects, which are crucial features for developing effective topical anti-inflammatory and anti-osteoarthritic therapies [4].

Therefore, in this study, we aim to develop and evaluate the in vitro anti-osteoarthritic and anti-inflammatory activities of Nyctanthes Arbor Tristis Linn Emulgel, which could serve as a promising alternative therapy for the management of OA [5].

Methodology

Formulation of Emulgel [6, 7]

This herbal formulation was prepared using different polymers such as Carbapol 934, Carbapol 940 and HPMC. 1% of the amount of the polymer were used and dissolved in the hot water (80^{o}C) using mechanical shaker and left overnight. The drug was thoroughly mixed in the water.

The Oil phase of the Emulgel was prepared using Tween 80. The Tween 80 was thoroughly mixed in the liquid paraffin wax. The oil phase was stored in separate container. The aqueous phase prepared using Tween 80 in the water. Along with that methyland propyl paraben were dissolving in Propylene glycol. The drug solution and the preservative solutions were added to the aqueous phase. Both the phases were heated and mixed together. The solution of the Emulsion and Gel mixed together at the ratio of 1:1.

The formulation was maintained the pH under the 7. The pH fluctuation was optimized by using the tri-ethanolamine. The optimized formulation was then mixed with the extract (1%). The formula followed as mentioned in the Table-1. The gel and formulation was thoroughly mixed using the magnetic stirrer at the constant temperature

Table- 1: Formulae of Emulgel

Sn.	Ingredients	Formulations			
		F1	F2	F3	F4
1	Extract	1	1	1	1
2	Carbopol 940	1	NA	NA	2
3	Carbopol 934	NA	1	NA	NA
4	HPMC	NA	NA	1	NA
5	Liquid paraffin	7.5	7.5	7.5	7.5
6	Propylene glycol	5	5	5	5
7	Methyl Parabene	0.03	0.03	0.03	0.03
8	Propyl Parabene	0.03	0.03	0.03	0.03
9	Water	q.s.	q.s.	q.s.	q.s.

Evaluation Parameters [8, 9]

The evaluation of a new Emulgel formulation was performed using various parameters such as physical examinations, determination of pH, viscosity, spreadability, in vitro drug release, drug content, and extrudability. The physical examination involved the observation of the formulation's texture, color, clarity, and the existence of any particles. The pH was measured using a digital pH meter, and the average value with standard deviation was recorded. Viscosity was measured at different RPM using a viscometer and the average reading was noted. Spreadability was evaluated by applying constant force to the formulation on glass plates, and the circle of the spread was noted down.

In vitro drug release was studied using the Franz diffusion cell apparatus, and the cumulative drug release was evaluated using UV-VIS spectrophotometry. Drug content was determined by diluting the sample in water, filtering it, and then analyzing the diluted content spectrophotometrically. Extrudability was evaluated using the percentage of the amount displaced by the amount forced out from the aluminum tubes. The Emulgel was tested triplicates, and the average data were compared with the standard formulation.

In vitro Evaluation [10, 11]

Cell Line Preparation

The RAW 264.7 cell line was grown in Dulbecco's Modified Eagle's Medium (DMEM) under 5% CO2 at 37°C with >90% humidity for 24 hours. After 48 hours, the cells were checked for confluency and appearance. The cells were then transferred into 96-well plates and exposed to 100 µl of test items, positive control, negative control, and blank in duplicates. Eight different concentrations (0.039 mg/mL, 0.078 mg/mL, 0.156 mg/mL, 0.312 mg/mL, 0.625 mg/mL, 1.25mg/mL, 2.5mg/mL, and 5 mg/mL) of the test item were added, and the concentration of the positive control was 5 mg/mL. The cells were further incubated in DMEM for 24 hours under 5% CO2 at 37°C with >90% humidity.

Observation

After 24 hours of treatment, the cells were examined using a phase-contrast microscope to check for morphological changes. The media was then replaced with fresh media containing MTT solution (5 mg/mL), and the plate was incubated for an additional 4 hours. After incubation, the media was replaced with fresh media, and the plates were placed in a microplate reader, and absorbance was measured at 570 nm wavelength. The absorbance was measured at 550nm using Biorad Microplate reader to ensure cell viability.

In Vitro Anti-Inflammatory Assay: The IL-6 release in RAW 264.7 cells was measured to evaluate the anti-inflammatory activity. After growing up the cells (70-80% confluency), they were removed from the culture flasks by enzymatic digestion (Trypsin/EDTA), and the cell suspension was centrifuged (1100-1200 rpm for 10 min). The cells were then re-suspended in culture medium at a density of 1-3 x 105cells/mL.

Test Item Exposure

A plate map (96-well microtitre plate) was prepared for blank, negative control, and test items. After 24 hours of incubation, 100 µl of test item at eight different concentrations (5 mg/mL) with LPS (1mg/mL), positive control (LPS, 5 mg/mL), negative control (cell alone), and blank (media only) were added in duplicates into the respective wells of the 96-well plate. The cells were further incubated for 24 hours at 5% CO2 at 37°C with >90% humidity.

Measurement of IL-6 Released [12, 13]

After 24 hours, the contents of each well were transferred to individually labeled 2 mL centrifuge vials. The contents were then centrifuged at 1100 rpm for 5 minutes, and the cell culture supernates were collected in a fresh labeled Eppendorf tube and stored in the refrigerator (2-8°C) for the estimation of the level of IL-6 released using an ELISA kit. The Human IL-6 ELISA Research Reagent procured from BOSTER was used for the IL-6 measurement as per ELISA manual. The assay samples were kept in 2-8°C until analysis.

Results and Discussion

Nyctanthes arbor-tristis leaves were collected and formulated as an Emulgel to evaluate its activities against arthritis and inflammation. The Emulgel was formulated using three different gelling agents, and various evaluation parameters were included in the study, such as organoleptic properties, homogeneity, pH determination, spreadability, viscosity, extrudability, in vitro drug release, and stability studies.

Phytochemical screening was performed to confirm the presence of phytochemicals in the extract of the leaves. The extract was identified in all three solvents, including methanol, ethanol, and water. The results showed the presence of alkaloids, carbohydrates, saponins, glycosides, steroids, phenols, and flavonoids, indicating the potential therapeutic effects of the Nyctanthes arbor-tristis extract.

The evaluation parameters were performed on both the gel and Emulgel formulations, and the results showed that the consistency and homogeneity of all four formulations were excellent and good, respectively. The pH of the Emulgel formulation fell within the acceptable range of 6.00 to 6.17. The viscosity of all four formulations remained constant in terms of consistency and fell within the range of 641.67 at 0.5 RPM and 407.2 at 1 RPM. The spreadability of the Emulgel formulation was satisfactory, with uniform spread observed in all four formulations when a constant force was applied. Formulation F2 showed the best spreadability among all four formulations.

The in vitro drug release from the Emulgel formulation was evaluated using the Franz Diffusion cell apparatus. The results showed that formulation F2 had the maximum drug release (95.66) among all other formulations. The drug content evaluation showed that formulation F2 had the maximum drug content (95.77) among all other Emulgel formulations.

Stability studies were performed on all four formulations, and the results showed that formulation F2 was stable and maintained its physical appearance during the study period of three months. The pH, spreadability, and drug content parameters were also stable during the stability studies. Overall, the F2 formulation showed remarkable stability and integrity, and consistently performed well in all evaluation parameters, making it the best among all four formulations.

The results from the experiment showed the viability of RAW 264.7 cell lines after exposure to test item TC at different concentrations. The data was presented in Table-2, including the negative control, positive control, blank, and test item at different concentrations. The optical density (OD) at 550nm was measured, and the percentage of viability was calculated using the given equation.

The negative control was considered 100% viable, and the percentage of viability for the other samples was calculated relative to the negative control. The percentage of viability decreased as the concentration of the test item increased, indicating that the test item had a cytotoxic effect on the RAW 264.7 cell lines.

The results showed that at concentrations of 5mg/mL and 2.5mg/mL, the viability was lower than 70% of the negative control, indicating cytotoxic potential. At concentrations lower than 2.5mg/mL, the viability was higher than 70% of the negative control, indicating no cytotoxicity.

In conclusion, the results from the experiment showed that the formulation 1 had no cytotoxic effect on RAW 264.7 cell lines at concentrations of 5mg/mL and 2.5mg/mL.

The analysis of Table-5 reveals several observations regarding the cytotoxic potential of the test substance. The blank values for both replicates are consistent and low, indicating that the assay was not affected by interference from the medium used. The negative control values are also consistent and fall within an acceptable range, indicating that the cells were viable and the assay was performed correctly. The positive control values are lower than the negative control values, demonstrating that the test substance at 5 mg/mL was not cytotoxic to the cells.

The test substance values show a dose-dependent decrease in viability compared to the negative control, indicating that the test substance has no cytotoxic potential. However, the viability values of TC1 to TC8 are all above 70% of the negative control, indicating that the cytotoxic potential of the test substance is not severe. Overall, the data suggests that the test substance has cytotoxic potential at higher concentrations but is not severely toxic to the cells at the tested concentrations

Table-6 presents the OD values and %Viability data for Formulation 3. The blank values for both replicates are consistent and low, indicating that there was no interference from the medium used in the assay. The negative control (NC) values are consistent between replicates and within an acceptable range, indicating that the assay was performed correctly and the cells were viable. The positive control (PC) values are lower than the negative control values, indicating that the test substance (5 mg/mL) was not cytotoxic to the cells. The test substance values (TC1 to TC8) show a dose-dependent decrease in viability compared to the negative control, indicating that the test substance has no cytotoxic potential. The viability values of TC1 to TC8 are all above 70% of the negative control.

Table-7 provides data on the viability of cells treated with Formulation 4 at various concentrations. The blank values for both replicates are consistent and low, indicating no interference from the medium used in the assay. The negative control (NC) values are consistent between replicates and within an acceptable range, indicating that the assay was performed correctly and the cells were viable. The positive control (PC) values are lower than the negative control values, indicating that the test substance (5 mg/mL) was cytotoxic to the cells. The test substance values (TC1 to TC8) show a dose-dependent decrease in viability compared to the negative control.

However, the viability values of TC1 to TC8 are all above 70% of the negative control, indicating that the cytotoxic potential of the test substance is not severe. Compared to the results of Formulation 1, 2 and 3, Formulation 4 shows a more pronounced decrease in viability at higher concentrations. For example, TC4 and TC5 of Formulation 4 have lower viability percentages compared to the same concentrations in Formulation 3.

Based on the statistical analysis, the concentration of the test item significantly influenced the release of IL-6 in the treated cells ($p < 0.05$). The mean concentration of IL-6 increased with increasing concentrations of the test item. F2 had the highest mean concentration of IL-6 compared to the other formulations, indicating that it had the strongest pro-inflammatory response. F1 had the lowest mean concentration of IL-6 among all the formulations.

It is important to note that the positive control had a higher mean concentration of IL-6 compared to all the test items, indicating that it was able to induce a stronger pro-inflammatory response. Meanwhile, the negative control

did not show any detectable level of IL-6, suggesting that the cells were not activated and did not produce any inflammatory response.

Tables- 2: Evaluation Parameters of Emulgel

Test	Formulations	Result
pH Determination	F1	6.17±0.12
	F2	6.17±0.25
	F3	6.27±0.21
	F4	6.00±0.10
Viscosity	F1	0.5 RPM: 582.87±5.44, 1 RPM: 309.33±2.30
	F2	0.5 RPM: 431.8±40.32, 1 RPM: 226.47±4.43
	F3	0.5 RPM: 607.53±4.82, 1 RPM: 304.67±3.41
	F4	0.5 RPM: 641.67±31.72, 1 RPM: 407.2±5.02
Spreadability	F1	10.59±0.56
	F2	12.49±0.45
	F3	9.87±0.68
	F4	8.92±0.68
Drug Content	F1	95.23±0.06
	F2	95.77±0.15
	F3	94.63±0.38
	F4	94.20±0.10
Extrudability	F1	Excellent
	F2	Excellent
	F3	Good
	F4	Good

Tables- 3: In vitro drug Release of Emulgel

Sn.	Time	F1	F2	F3	F4
1	0	0	0	0	0
2	1	11.01	10.25	12.24	11.21
3	2	28.21	30.11	27.25	26.54
4	3	35.02	39.25	30.28	32.65
5	4	51.27	59.82	50.13	49.39
6	5	59.88	61.34	60.89	58.65
7	6	62.41	72.98	68.32	65.02
8	7	78.96	85.25	78.32	70.06
9	8	90.00	95.66	89.69	88.35

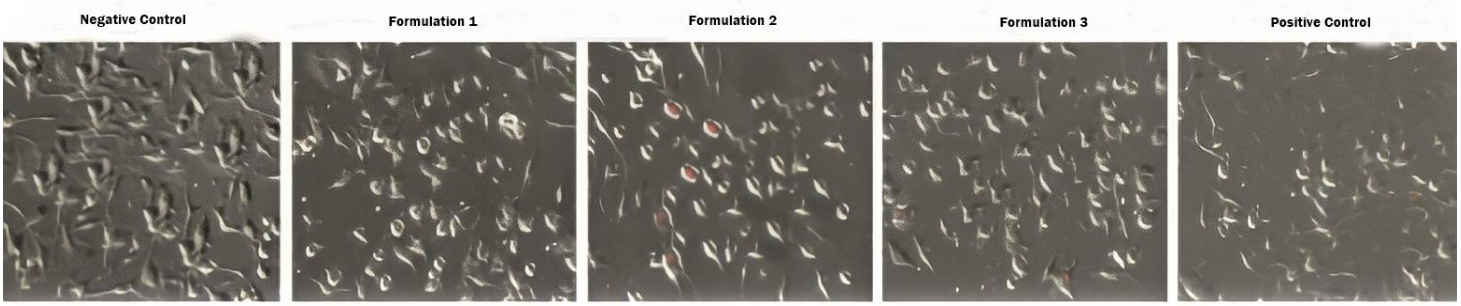
Table-4: Plate Mapping for Cell Seeding and Test Item Exposure for Experiments (RAW 264.7Cell lines)

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	NC	P C	TC-5 mg/mL	TC - 2.5mg/ mL	TC-1.25 mg/mL	TC- 0.625mg/ mL	TC 0.313m g/mL	TC- 0.156mg/ mL	TC- 0.078mg/ mL	TC- 0.039m g/mL	
B	Blank	NC	P C	TC -5 mg/mL	TC- 2.5mg/ mL	TC-1.25 mg/mL	TC- 0.625mg/ mL	TC 0.313m g/mL	TC- 0.156mg/ mL	TC- 0.078mg/ mL	TC- 0.039m g/mL	
C												
D												
E												
F												
G												
H												

Note: Negative control,P-Positive Control (5 mg/ml), Test itemTC1 to TC2(concentrations of 5mg/mL, 2.5mg/mL, 1.25mg/mL, 0.625mg/mL, 0.313mg/mL, 0.156mg/mL, 0.078mg/mL and 0.039mg/mL), shaded wells were unused.

Table-5: OD vs. %Viabilityfor Formulation 1

ID	Conc. (mg/mL)	OD I	OD II	Mean OD	SD	%CV	%Viability
Blank	NA	0.0547	0.0546	0.054665	9.192391223	0.1682	NA
NC	NA	0.1183	0.1178	0.11805	0.000353553	0.2995	100
PC	5	0.0667	0.0654	0.06605	0.000919239	1.3917	55.95
TC1	5	0.0915	0.0942	0.09285	0.001909188	2.0562	78.65
TC2	2.5	0.0952	0.093	0.0941	0.001555635	1.6532	79.71
TC3	1.25	0.0976	0.0961	0.09685	0.00106066	1.0952	82.04
TC4	0.625	0.0989	0.0995	0.0992	0.000424264	0.4277	84.03
TC5	0.312	0.1031	0.1019	0.1025	0.000848528	0.8278	86.83
TC6	0.156	0.1054	0.1044	0.1049	0.000707107	0.6741	88.86
TC7	0.078	0.1072	0.1075	0.10735	0.000212132	0.1976	90.94
TC8	0.039	0.1087	0.1093	0.109	0.000424264	0.3892	92.33



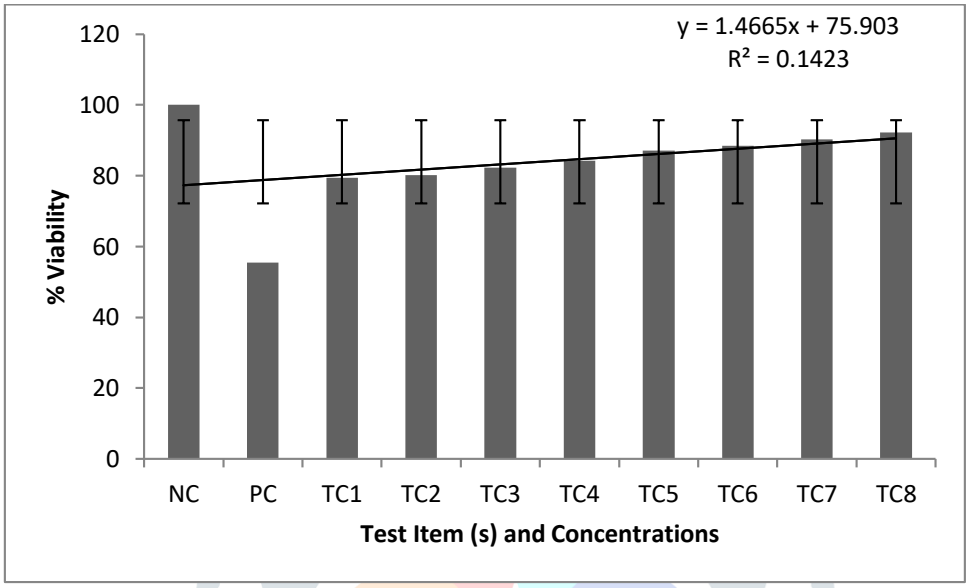


Fig.1- OD vs. %Viabilityfor Formulation 1

Table-6: OD vs. %Viabilityfor Formulation 2

ID	Conc. (mg/mL)	OD I	OD II	Mean OD	SD	%CV	%Viability
Blank	NA	0.0584	0.0588	0.0586	0.000282843	0.4827	NA
NC	NA	0.1221	0.1201	0.1211	0.001414214	1.1678	100
PC	5	0.0709	0.0699	0.0704	0.000707107	1.0044	58.13
TC1	5	0.0895	0.0892	0.08935	0.000212132	0.2374	73.78
TC2	2.5	0.0917	0.0899	0.0908	0.001272792	1.4018	74.98
TC3	1.25	0.0942	0.0945	0.09435	0.000212132	0.2248	77.91
TC4	0.625	0.0965	0.0959	0.0962	0.000424264	0.441	79.44
TC5	0.312	0.1007	0.0991	0.0999	0.001131371	1.1325	82.49
TC6	0.156	0.1019	0.1022	0.10205	0.000212132	0.2079	84.27
TC7	0.078	0.1071	0.1062	0.10665	0.000636396	0.5967	88.07
TC8	0.039	0.1118	0.1107	0.11125	0.000777817	0.6992	91.87

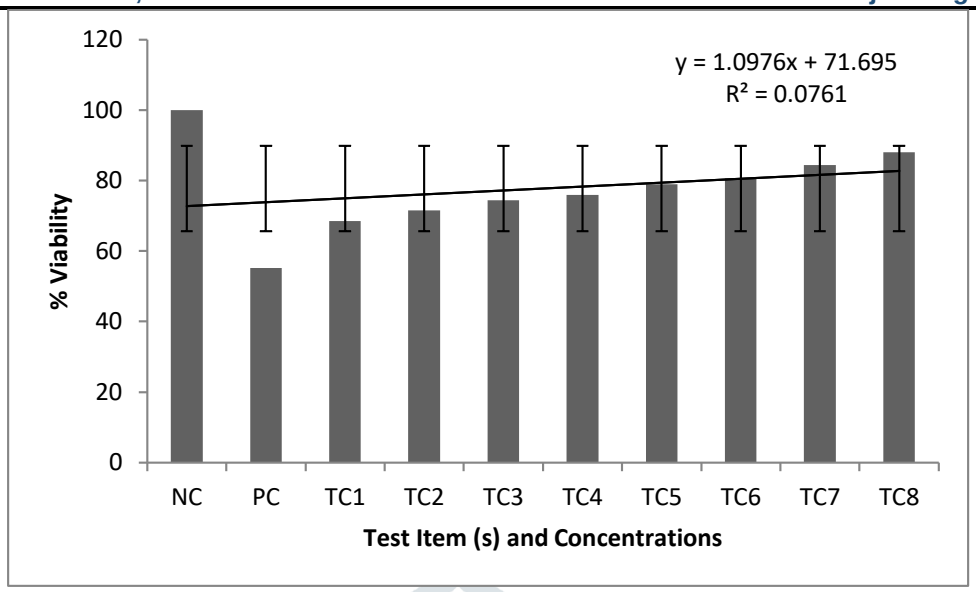


Fig.2- OD vs. %Viabilityfor Formulation 2

Table-7: OD vs. %Viabilityfor Formulation 3

ID	Conc. (mg/mL)	OD I	OD II	Mean OD	SD	%CV	%Viability
Blank	NA	0.0520	0.0512	0.0516	0.000565685	1.0963	NA
NC	NA	0.1231	0.1235	0.1233	0.000282843	0.2294	100
PC	5	0.0628	0.0619	0.06235	0.000636396	1.0207	50.65
TC1	5	0.0915	0.0911	0.0913	0.000282843	0.3098	74.17
TC2	2.5	0.0929	0.0927	0.0928	0.000141421	0.1524	75.39
TC3	1.25	0.0931	0.0929	0.093	0.000141421	0.1521	75.55
TC4	0.625	0.0955	0.0962	0.09585	0.000494975	0.5164	77.86
TC5	0.312	0.0978	0.0988	0.0983	0.000707107	0.7193	79.85
TC6	0.156	0.1019	0.1025	0.1022	0.000424264	0.4151	83.02
TC7	0.078	0.103	0.1034	0.1032	0.000282843	0.2741	83.83
TC8	0.039	0.1171	0.1169	0.117	0.000141421	0.1209	95.04

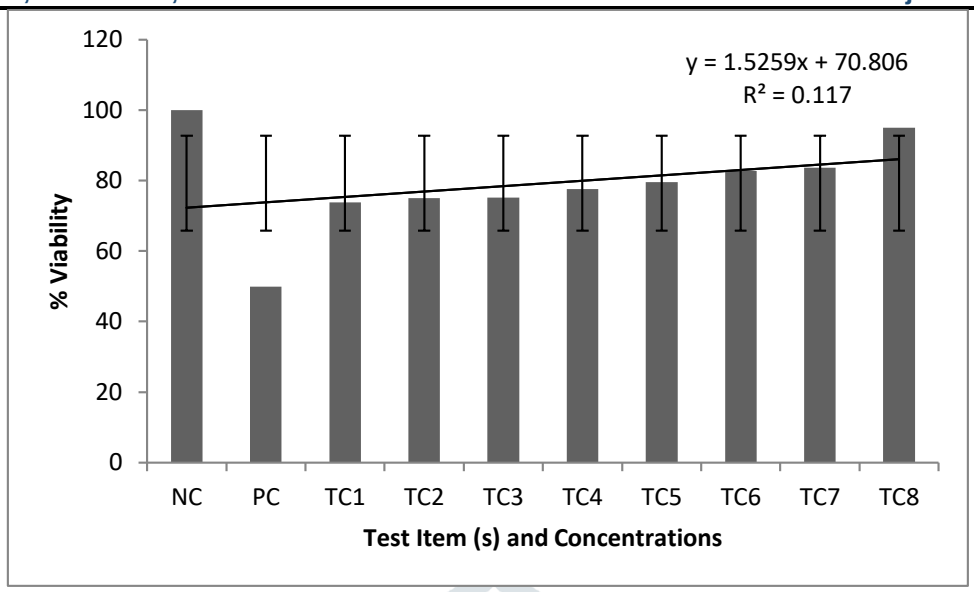


Fig.3- OD vs. %Viabilityfor Formulation 3

Table-8: OD vs. %Viabilityfor Formulation 4

ID	Conc. (mg/mL)	OD I	OD II	Mean OD	SD	%CV	%Viability
Blank	NA	0.0532	0.0537	0.05345	0.000353553	0.6615	NA
NC	NA	0.1361	0.1331	0.1346	0.00212132	1.576	100
PC	5	0.0638	0.0628	0.0633	0.000707107	1.1171	47.03
TC1	5	0.1005	0.1007	0.1006	0.000141421	0.1406	74.74
TC2	2.5	0.1019	0.1017	0.1018	0.000141421	0.1389	75.63
TC3	1.25	0.1044	0.1037	0.10405	0.000494975	0.4757	77.30
TC4	0.625	0.1144	0.121	0.1177	0.004666905	3.9651	87.44
TC5	0.312	0.1149	0.1152	0.11505	0.000212132	0.1844	85.48
TC6	0.156	0.1177	0.1189	0.1183	0.000848528	0.7173	87.89
TC7	0.078	0.1181	0.1188	0.11845	0.000494975	0.4179	88.00
TC8	0.039	0.1198	0.1202	0.12	0.000282843	0.2357	89.15

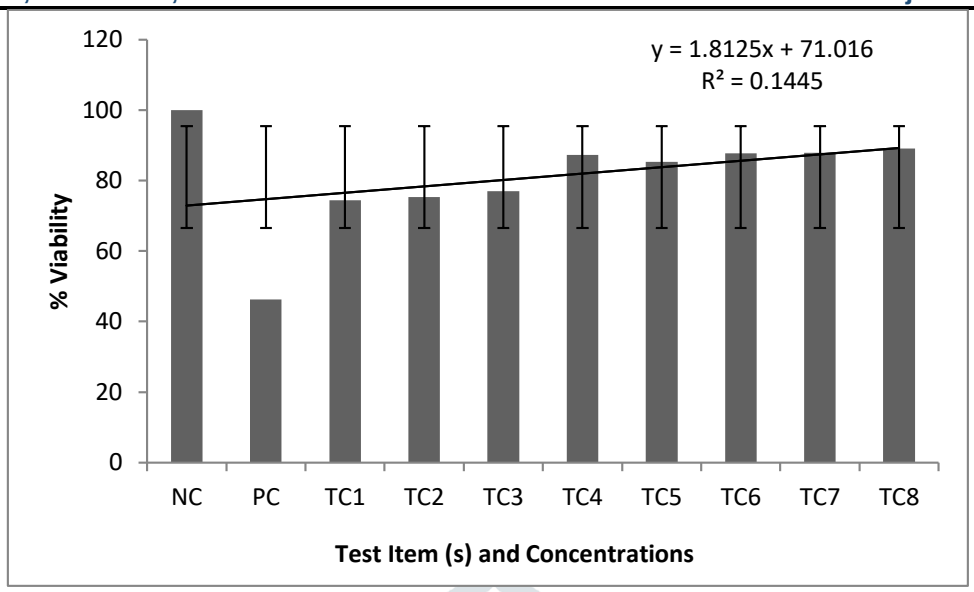


Fig.4- OD vs. %Viabilityfor Formulation 3

Table-9: Plate Map for the Determination of IL-6

	1	2	3	4	5	6	7	8	9	10	11	12
a	AC	AC	5mg/ml	5mg/ml	NC							
b	Std1	Std1	2.5mg/ml	2.5mg/ml	NC							
c	Std2	Std2	1.25mg/ml	1.25mg/ml	PC							
d	Std3	Std3	0.625mg/ml	0.625mg/ml	PC							
e	Std4	Std4	0.313mg/ml	0.313mg/ml								
f	Std5	Std5	0.156mg/ml	0.125mg/ml								
g	Std6	Std6	0.078mg/ml	0.0625mg/ml								
h	Std7	Std7	0.039mg/ml	0.031mg/ml								

Note: AC-Assay Control (sample diluents buffer), RC-Reagent Control (DMEM medium), Std-Standard 1-7 is 300, 150, 75, 37.5, 18.75, 9.38, 4.69 pg/ml, 5 mg to 0.039 Test Item concentrations treated cell supernatant, NC-Negative Control, PC-Positive Control, Columns 3-4- Test Item-1, Columns 5-6- Test Item-2. Shaded wells are unused wells

Table 10:IL-6 concentration inTest item Treated Cells

ID	F1	F2	F3	F4
NC	NA	NA	NA	NA
PC	-	-	-	-
TC8- 0.039mg/ml	25.281	35.388	42.905	32.662
TC7- 0.078 mg/ml	28.462	38.680	43.390	36.218
TC6- 0.156 mg/ml	43.163	51.114	49.141	43.499
TC5- 0.313 mg/ml	44.380	53.012	52.394	47.171
TC4- 0.625 mg/ml	57.702	62.612	55.747	53.979
TC3- 1.25 mg/ml	59.551	68.484	65.143	62.411
TC2- 2.5 mg/ml	61.662	70.959	68.709	63.913
TC1- 5 mg/ml	66.894	74.568	73.850	67.511

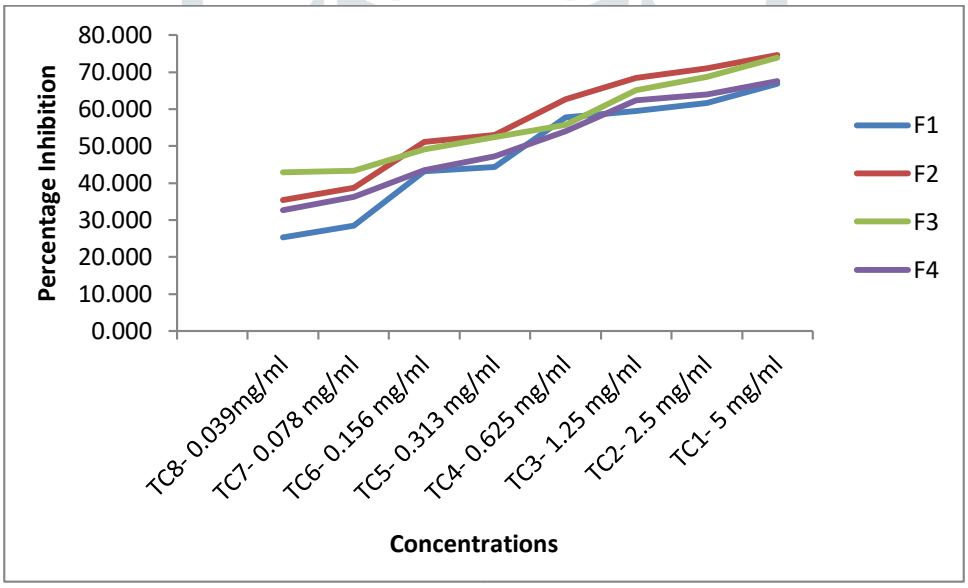


Fig.5- Formulations vs. % Inhibition

Conclusion

The results of the experiment showed that the test items had no cytotoxic potential on RAW 264.7 cell lines at higher concentrations of 5mg/mL and 2.5mg/mL, while lower concentrations also did not exhibit cytotoxicity. The analysis of Tables 3, 4, and 5 showed that the test substance had a dose-dependent decrease in viability compared to

the negative control, indicating that the cytotoxic potential of the test substance is not severe. However, Formulation 4 showed a more pronounced decrease in viability at higher concentrations compared to the other formulations. Overall, the data suggests that the test substance has no cytotoxic potential at higher concentrations.

The statistical analysis showed that the concentration of the test item significantly influenced the release of IL-6 in the treated cells. F2 had the highest mean concentration of IL-6 compared to the other formulations, indicating that it had the strongest pro-inflammatory response. Meanwhile, F1 had the lowest mean concentration of IL-6 among all the formulations.

In conclusion, the experiment provided evidence of the cytotoxic and pro-inflammatory potential of the formulation 1-4 on RAW 264.7 cell lines. The results suggest that the test substance has no cytotoxic potential at higher concentrations. The statistical analysis also demonstrated that the concentration of the test item significantly influenced the release of IL-6 in the treated cells, indicating its pro-inflammatory potential.

References

1. Boer, C. G., Hatzikotoulas, K., Southam, L., Stefánsdóttir, L., Zhang, Y., de Almeida, R. C., ... & Wilkinson, J. M. (2021). Deciphering osteoarthritis genetics across 826,690 individuals from 9 populations. *Cell*, *184*(18), 4784-4818.
2. Parul Nigam, Prateek Mishra, & Sneha Yadav. (2023). Formulation and Development of Boswellia serrata Extract-Loaded Microspheres for Targeted Delivery of Anti-Inflammatory Agents. PEXACY International Journal of Pharmaceutical Science, 3(1), 1–13. https://doi.org/10.5281/zenodo.7690671.
3. Gato-Calvo, L., Magalhaes, J., Ruiz-Romero, C., Blanco, F. J., & Burguera, E. F. (2019). Platelet-rich plasma in osteoarthritis treatment: review of current evidence. *Therapeutic advances in chronic disease*, *10*, 2040622319825567.
4. Khan, B. A., Ullah, S., Khan, M. K., Alshahrani, S. M., & Braga, V. A. (2020). Formulation and evaluation of Ocimum basilicum-based emulgel for wound healing using animal model. *Saudi pharmaceutical journal*, *28*(12), 1842-1850.
5. Bari, M. S., Das, A. C., Bhuiyan, M. M. U., Khatun, S., Mitu, A., & Khandokar, L. (2022). Antioxidant, anti-inflammatory, thrombolytic, and cytotoxic activities of Gynura nepalensis DC. and isolation of its steroid constituents. *Indian Journal of Natural Products and Resources (IJNPR)[Formerly Natural Product Radiance (NPR)]*, *13*(3), 329-338.
6. Khan, B. A., Ullah, S., Khan, M. K., Alshahrani, S. M., & Braga, V. A. (2020). Formulation and evaluation of Ocimum basilicum-based emulgel for wound healing using animal model. *Saudi pharmaceutical journal*, *28*(12), 1842-1850.
7. Burki, I. K., Khan, M. K., Khan, B. A., Uzair, B., Braga, V. A., & Jamil, Q. A. (2020). Formulation development, characterization, and evaluation of a novel dexibuprofen-capsaicin skin emulgel with improved in vivo anti-inflammatory and analgesic effects. *AAPS PharmSciTech*, *21*, 1-14.

8. Yousuf, M., Khan, H. M. S., Rasool, F., Khan, K. U. R., Usman, F., Ghalloo, B. A., ... & Conte-Junior, C. A. (2022). Chemical profiling, formulation development, in vitro evaluation and molecular docking of Piper nigrum Seeds extract loaded Emulgel for anti-Aging. *Molecules*, *27*(18), 5990.
9. Gusai, T., Dhavalkumar, M., Soniwala, M., Dudhat, K., Vasoya, J., & Chavda, J. (2021). Formulation and optimization of microsponge-loaded emulgel to improve the transdermal application of acyclovir—a DOE based approach. *Drug Delivery and Translational Research*, *11*, 2009-2029.
10. Omar, M. M., Eleraky, N. E., El Sisi, A. M., & Ali Hasan, O. (2019). Development and evaluation of in-situ nasal gel formulations of nanosized transferosomal sumatriptan: Design, optimization, in vitro and in vivo evaluation. *Drug design, development and therapy*, 4413-4430.
11. Bhatt, P., Narvekar, P., Lalani, R., Chougule, M. B., Pathak, Y., & Sutariya, V. (2019). An in vitro assessment of thermo-reversible gel formulation containing sunitinib nanoparticles for neovascular age-related macular degeneration. *AAPS PharmSciTech*, *20*, 1-14.
12. Pal, R. R., Rajpal, V., Singh, N., Singh, S., Mishra, N., Singh, P., ... & Saraf, S. A. (2023). Downregulation of pro-inflammatory markers IL-6 and TNF-α in rheumatoid arthritis using nano-lipidic carriers of a quinone-based phenolic: an in vitro and in vivo study. *Drug Delivery and Translational Research*, *13*(2), 627-641.
13. Pal, R. R., Rajpal, V., Singh, N., Singh, S., Mishra, N., Singh, P., ... & Saraf, S. A. (2023). Downregulation of pro-inflammatory markers IL-6 and TNF-α in rheumatoid arthritis using nano-lipidic carriers of a quinone-based phenolic: an in vitro and in vivo study. *Drug Delivery and Translational Research*, *13*(2), 627-641.